

# **A Novel Attenuation Mechanism of Zinc within Aquatic Plants: Complexation on Root Borne Manganese Nodules**

C.M. Hansel<sup>1</sup>, S.E. Fendorf<sup>1</sup>, and G.M. Lamble<sup>2</sup>

<sup>1</sup>Dept. of Geological and Environmental Sciences, Stanford University, Palo Alto, CA 94305

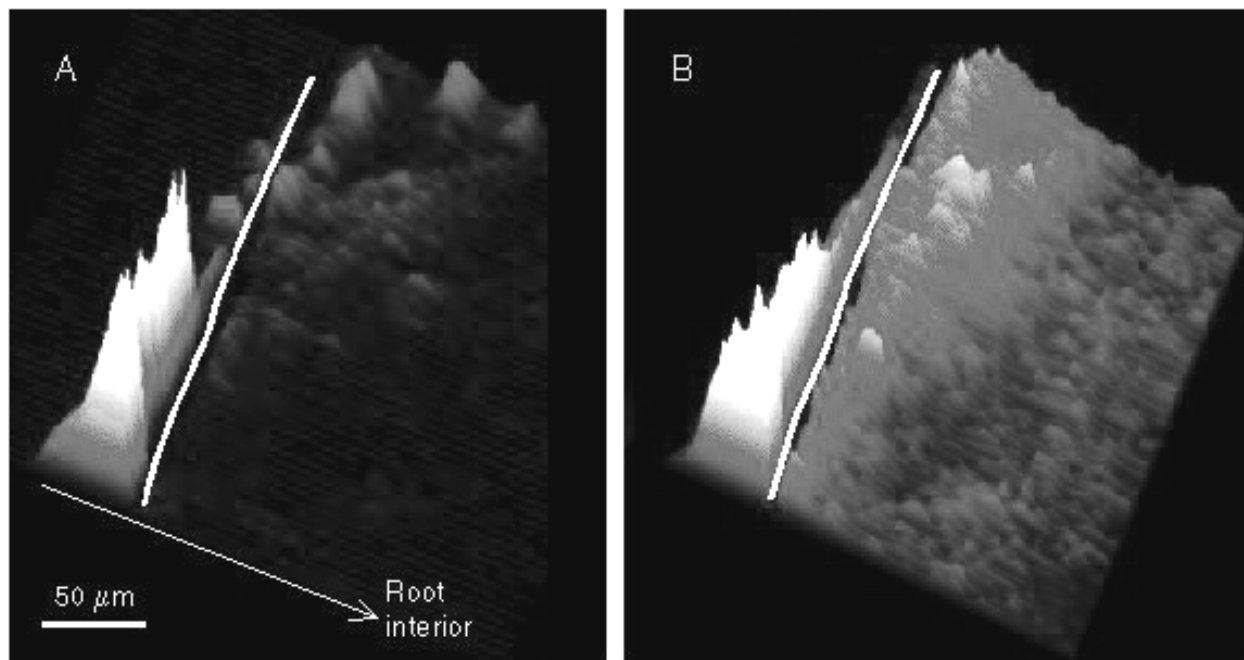
<sup>2</sup>Advanced Light Source, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720

The ability of plants to grow and prosper in contaminated soils of contrasting properties is intriguing yet enigmatic. Aquatic plants, such as those within wetlands, have in particular been recognized for their ability to sequester trace element contaminants [1]. Identification of direct mechanisms involved in plant accumulation and tolerance is essential not only for discerning physiological adaptations by plants to metal contaminated soils but also for exploiting specific plant attributes to maximize efficiency in metal binding. Furthermore, the specific binding mechanisms of trace elements within plants has important implications for the delineation of potential bioavailability and/or release of toxins upon changing physicochemical conditions within aquatic environments. While accumulation of metals in the shoots of plants has been explored extensively [2], the role of plant roots is ill defined despite their ability to sequester toxins at high levels. Iron (hydr)oxide coatings, or plaque, formed on plant roots are suspected to directly hinder the uptake and translocation of phytotoxins [3]; however, the direct mechanisms of metal immobilization have yet to be determined. Moreover, manganese has been given only cursory attention although it is a ubiquitous, redox sensitive element that forms reactive (hydr)oxides as well. Here we describe the critical and unforeseen role of manganese in contaminant attenuation and cycling within aquatic plants. The formation of discrete manganese oxide nodules on root surfaces of aquatic plants, which subsequently induced local sequestration of zinc (an acute toxin of aquatic organisms), was revealed using x-ray microprobe analysis.

The novel technology of phytoremediation has prompted countless studies to determine optimal species for maximal accumulation [4], chemical modifications to improve accumulation and translocation [5] and, most recently, the mechanisms allowing for this phenomenon [6-7]. Due in part to the modern emergence of synchrotron radiation techniques (e.g., x-ray absorption fine structure spectroscopy), metal speciation in plant biomass is currently being ascertained [7]. Recent studies, however, focus on aerial portions of plant-metal complexation yet contaminant concentrations in the roots are generally several fold higher. Accordingly, our research focuses on the mechanisms underlying the sequestration of toxins within the rhizosphere of aquatic plants.

A number of grass species (e.g., reed canarygrass) have demonstrated tolerance and accumulation of heavy metals [8]. *Phalaris arundinacea* L. (reed canarygrass) belongs to the grass family and is indigenous to aquatic environments. Accordingly, reed canarygrass samples underwent acid digestions, which revealed extensive concentrations of metals and metalloids including As, Fe, Mn, Pb and Zn. Consistent with previous investigations, concentrations of trace elements (As, Pb, Zn) in the plant roots are several fold higher than in shoots. Root tissue concentrations for As, Fe, Mn, Pb and Zn are 96.2, 15100, 1500, 892, and 1050-mg kg<sup>-1</sup>, respectively. Scanning electron microscopy (SEM) discloses the extensive nature of a plaque on the exterior of the grass roots (data not shown). Coatings exist predominantly as casts or fillings in cavities of the epidermal cells, which are composed predominantly of Fe with lesser amounts of As, Mn, Pb and Zn.

A cross-sectional map from the interior of the root through the epidermis and plaque was constructed for manganese and zinc using a synchrotron based x-ray microprobe (Fig. 1). Scans



**Figure 1.** Microprobe map of Mn and Zn concentration distributions on and within grass roots. White line represents the root epidermis and relative elemental concentrations increase with color brightness. (A) Spatial distribution of Mn along a transect from root center to exterior of plaque. (B) Spatial distribution of Zn along the same transect as that for Mn.

were performed on beamline 10.3.2 at the Lawrence Berkeley National Laboratory Advanced Light Source (LBNL-ALS). The scan covers a 250 x 250-μm section of the root with data collection at 5-μm increments. The two elements were mapped simultaneously along the same transect such that direct spatial associations and distributions were ascertained. The distribution of Mn is concentrated in discrete oxidized zones on the exterior of the epidermis. Concentrations of Mn diminish in areas adjacent to these zones and are negligible in the interior of the root. The spatial distribution of Zn on grass roots corresponds remarkably with the distribution patterns for Mn. Zinc concentrations are elevated at the discrete sites of Mn deposition, while concentrations decrease elsewhere. Concentrations of Zn and Mn are strongly correlated aside from the interior cell wall where Zn concentrations are slightly higher.

The morphology of the Mn-Zn precipitate on the plant root is analogous to that of seafloor ferromanganese nodules. Nodules have been discovered in a variety of environments including marine and freshwater systems, soils, desert varnish and streambeds [9]. Seafloor ferromanganese nodule formation is initiated by precipitation of a hydrated iron oxide on a nucleating agent (pumice, rocks, glass, clays) followed by cyclic deposition of Mn oxides [10]. Structural similarities between iron and manganese (hydr)oxides, such as ferrihydrite and birnessite, make them amenable to epitaxial growth thus allowing for nodule formation and oscillatory intergrowths of Fe and Mn bearing minerals. The specific and high affinity of birnessite for contaminants such as cadmium and zinc is well documented [11]. Zinc, although

an essential micronutrient to most biota, is a ubiquitous contaminant and toxic when present at excessive levels. Similarly, as a result of radial oxygen diffusion, plant roots serve as nucleating agents for the deposition of a hydrated iron hydroxide plaque, which subsequently provides a reactive substrate for manganese oxidation and precipitation. Consequently, discrete manganese nodules are produced on the root surface by way of epitaxial growth and auto-catalysis and may then provide a surface for attenuation of toxins such as zinc.

In conclusion, our research introduces and elucidates an unprecedented process behind the unique ability of aquatic plants to accumulate trace elements. A crucial and unforeseen mechanism underlying this phenomenon involves the formation of manganese nodules on the root epidermis and subsequent sequestration of zinc. Accordingly, the expansive distribution of ferromanganese nodules may now be augmented to include microscopic, and perhaps macroscopic, nodules on the epidermis of plant roots. Additionally, the ability for plant roots to serve as nucleating agents for hydroxide precipitation and nodule formation, as well as contaminant sequestration, has been introduced. Therefore, contrary to current belief [12], Zn attenuation on plant roots may be predominantly due to specific adsorption on to Mn oxide nodules rather than adsorption onto Fe oxides.

## ACKNOWLEDGEMENTS

We gratefully acknowledge M.J. La Force and G.C. Li for help with sample collection and analysis. We also thank LBNL-ALS for granted beam time.

## REFERENCES

1. G.J. Taylor and A.A. Crowder, *Can. J. Bot.* **61**, 1825 (1983); S.K. Jain, P. Vasudevan, N.K. Jha, *Biol. Wastes* **28**, 115 (1989); S.C. Mo, D.S. Choi, J.W. Robinson, *J. Environ. Sci. Health A24*, 135 (1989); Y.L. Zhu; A.M. Zayed, J-H. Qian, M. de Souza, N. Terry, *J. Environ. Qual.* **28**, 339 (1999).
2. A.W. McIntosh, B.K. Shephard, R.A. Mayes, G.J. Atchison, D.W. Nelson, *J. Environ. Qual.* **7(3)**, 301 (1978); A. Mudroch and J.A. Capobianco, *Hydrobiologia* **64(3)**, 223 (1979).
3. W. Armstrong, in *Plant Life in Anaerobic Environments*, D.E. Hook and R.M.M. Crawford, Eds. (Ann Arbor Science, Michigan, 1978), pp. 269-298; I.A. Mendelssohn and M.T. Postek, *Amer. J. Bot.* **69(6)**, 904 (1982).
4. P.B.A.N. Kumar, S. Dushenkov, H. Motto, I. Raskin, *Environ. Sci. Technol.* **29**, 1232 (1995); V. Dushenkov, P.B.A.N. Kumar, H. Motto, I. Raskin, *Environ. Sci. Technol.* **29**, 1239 (1995).
5. J.W. Huang, J. Chen, W.R. Berti, S.D. Cunningham, *Environ. Sci. Technol.* **31**, 800 (1997); M.J. Blaylock et al., *Environ. Sci. Technol.* **31**, 860 (1997).
6. L.R. Drake, S. Lin, G.D. Rayson, P.J. Jackson, *Environ. Sci. Technol.* **30**, 110 (1996).
7. C.M. Lytle, F.W. Lytle, B.N. Smith, *J. Environ. Qual.* **25**, 311 (1996); D.E. Salt et al., *Environ. Sci. Technol.* **31**, 1636 (1997); C.M. Lytle et al., *Environ. Sci. Technol.* **32**, 3087 (1998); K.J. Tiemann et al., *Environ. Sci. Technol.* **33**, 150 (1999).
8. R.J. Bartlett, *Soil Science* **92**, 372 (1961); E.K. Porter and P.J. Peterson, *Environ. Pollut.* **14**, 255 (1977).
9. J.D. Hem, *Geochim. Cosmochim. Acta* **45**, 1369 (1981).
10. R.G. Burns and V.M. Burns, *Nature* **255**, 130 (1975).

11. J.W. Murray, *Geochim. Cosmochim. Acta* **39**, 505 (1975); R.M. McKenzie, *Geochim. Cosmochim. Acta* **43**, 1855 (1979); R.J. Zasoski and R.G. Burau, *Soil Sci. Soc. Am. J.* **52**, 81 (1988).
12. M.L. Otte, J. Rozema, L. Koster, M.S. Haarsma, R.A. Broekman, *New Phytol.* **111**, 309 (1989); M.O. Boyle and M.L. Otte, *Environ. Pollut.* **96**, 1 (1997).

This research was supported by the Exploratory Research Program of the U.S. EPA.

Principal investigator: Scott Fendorf, Dept. of Geological and Environmental Sciences, Stanford University  
Email: fendorf@stanford.edu